BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

PETITION TO MAKE SPECIAL, REQUESTING THE ADVANCEMENT OF EXAMINATION UNDER 37 C.F.R. § 1.102 AND M.P.E.P. § 708.02(II),

First named inventor	:	Daly, John
App. No.	:	10/658,093
Filed	:	September 9, 2003
Title	:	CONSTRUCTS FOR GENE EXPRESSING ANALYSIS
Examiner	:	Unknown

Group Art Unit: Unknown

I hereby certify that this correspondence and all marked attachments are being deposited with the United States Postal Service as first-class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on

March 10, 2004
(Date)

Jennifer A. Haynes, Ph. D., Reg. No. 48,868

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

ATTN: Mail Stop DAC

Dear Sir:

Pursuant to 37 C.F.R. § 1.102, and M.P.E.P. § 708.02(II), Applicants hereby petition that the above application be made special due to actual infringement.

1. **A check in the amount of $130 to cover the petition fee is enclosed herewith.**

   Please charge any additional fees or credit overpayment to Deposit Account No. 11-1410.

2. **There is an infringing device or product actually on the market.**

The Applicant has discovered that Promega Corporation ("Promega") is selling vectors under the product name "Rapid Response™ Reporter Vectors", which fall within the scope of the pending claims. From the information available on their website (http://www.promega.com/newprod/, Promega lists this product under the subheading 'PROTEOMICS'. Activation of the hyperlink associated with the product name "Rapid Response™ Reporter Vectors" redirects the viewer to a webpage that describes these vectors in more detail, and that provides a price list and conditions for sale of those vectors. **A Technical Manual (#TM242)[Appendix A]** is listed on this webpage whose hyperlink redirects the viewer to an electronic copy of this manual. Hard copies of the web pages and technical manual are enclosed as Appendix A.

3. **A rigid comparison of this alleged infringing device or products with the claims has been made. In my opinion some of the claims are unquestionably infringed.**

The enclosed technical manual shows that vectors pGL3 (R2.2)-basic and phRG(R2.2)-basic each comprise a reporter gene operably linked to nucleic acid sequences encoding a protein degradation sequence and an mRNA degradation sequence (see page 3 of the Manual). The inventors have informed us that the intracellular half-life of the reporter protein expressed from these vectors is less than 1 hour. Accordingly, it is our opinion that these vectors fall within the scope of several of the claims.

4. A careful and thorough search of the prior art has been made.

A pre-examination search has been made of the presently claimed invention by the Australian Intellectual Property Office acting as the International Searching Authority for the international application of which the present application is a continuation-in-part. The results of this search are reported in the International Search Report for this international application. A copy of the **International Search Report [Appendix B]** and the documents cited are enclosed in the accompanying Information Disclosure Statement.

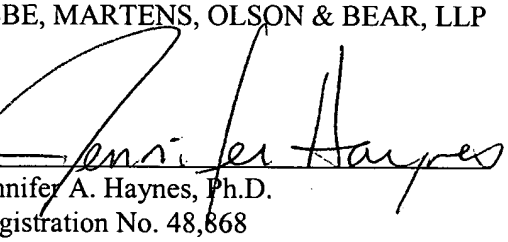
5. An Information Disclosure Statement and PTO form 1449 with 9 references enclosed.

6. A return prepaid postcard is enclosed.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: March 10, 2004

By: 

Jennifer A. Haynes, Ph.D.
Registration No. 48,868
Agent of Record
Customer No. 20,995
(415) 954-4114

W:\DOCS\JAH\JAH-7077.DOC
022604

Rapid Response™ Reporter Vectors

Promega

Technical Manual No. 242

INSTRUCTIONS FOR USE OF PRODUCTS E6431, E6441, E6451 AND E6461
All technical literature is available on the Internet at www.promega.com
Please visit the web site to verify that you are using the most current version of this Technical Manual.

I. Description

Firefly and *Renilla* luciferases are widely used to monitor transcriptional activity in cell biology and drug discovery applications because they are highly sensitive, flexible and easily quantified. However, these reporter proteins are relatively stable (Table 1) in mammalian cells and thus typically require several hours of incubation in order to detect changes in transcriptional activities. To reduce incubation times and shorten the time between transcription and reporter detection, Promega has designed luciferase genes that encode reporter proteins with reduced half-lives. The proteins encoded by these Rapid Response™ luciferase genes respond more quickly and with greater magnitude to changes in transcriptional activity than their more stable predecessors.

This Technical Manual provides information on the use of four Rapid Response™ Reporter Vectors[a,b,c]—the pGL3(R2.1)-Basic[a,b,c,d,e], pGL3(R2.2)-Basic[a,b,c,d,e], phRG(R2.1)-Basic[a,b,c,f] and phRG(R2.2)-Basic[a,b,c,f] Vectors. The pGL3(R2.1) and pGL3(R2.2) Vectors encode derivatives of firefly luciferase, and the phRG(R2.1) and phRG(R2.2) Vectors encode derivatives of *Renilla* luciferase. The R2.1 vectors (pGL3(R2.1)-Basic and phRG(R2.1)-Basic) encode reporter proteins that have reduced half-lives. The R2.2 vectors (pGL3(R2.2)-Basic and phRG(R2.2)-Basic) possess elements that reduce mRNA and protein half-lives.

Promega Corporation · 2800 Woods Hollow Road · Madison, WI 53711-5399 USA · Toll Free in USA 800-356-9526 · Telephone 608-274-4330 · Fax 608-277-2516 · www.promega.com
Printed in USA.
12/03
Part# TM242

Promega

A promoter or response element is required for reporter gene expression, thus one pertinent to your studies must be cloned into the Rapid Response™ Vectors.

Usage Information

The Rapid Response™ Reporter Vector backbone is Promega's pGL3-Basic[d,g] Vector, which does not contain promoter elements. A promoter or response element is required for reporter gene expression, thus one pertinent to your studies must be cloned into the Rapid Response™ Vectors. Use of the Rapid Response™ Reporter Vectors with Promega's firefly and *Renilla* Luciferase Assay Systems and reagents requires optimization.

II. Product Components

Product	Size	Cat.#
pGL3(R2.1)-Basic Vector[a,b,c,d,e]	20µg	E6431
pGL3(R2.2)-Basic Vector[a,b,c,d,e]	20µg	E6441
phRG(R2.1)-Basic Vector[a,b,c,f]	20µg	E6451
phRG(R2.2)-Basic Vector[a,b,c,f]	20µg	E6461

Storage Conditions: Store the Rapid Response™ Reporter Vectors at –20°C.

III. General Considerations

A. Characteristics of the Rapid Response™ Luciferase Genes

Firefly and *Renilla* luciferases are monomeric proteins that do not require post-translational modification for activity. Therefore, these enzymes can function as genetic reporters immediately following translation. Computational models predict that genetic reporters with reduced intracellular stability will yield faster response to changes in transcriptional rate and an increase in the relative magnitude of the response (1). Destabilized reporter proteins (i.e., those with faster protein degradation rates) are therefore expected to be more responsive and better suited to monitor rapid processes (such as promoter activation and repression) than reporters with slower protein degradation rates.

Transcription Factor Binding Sites Removed and Codon Usage Optimized

Native sequences for firefly and *Renilla* luciferase contain multiple consensus sequences for mammalian transcription factor binding sites. To increase the expression and reliability of Rapid Response™ Reporter Vectors, the luciferase genes present in these vectors were systematically redesigned, removing most of the consensus sequences for mammalian transcription factor binding sites. Additionally, the codons in both firefly and *Renilla* luciferase reporter genes were optimized for use in mammalian cells, creating the synthetic *hluc+* and *hRluc* genes, respectively.

Degradation Sequences Added

To generate reporter proteins that have increased protein degradation rates (i.e., destabilized reporters) two different degradation sequences have been incorporated into the synthetic firefly and *Renilla* luciferase genes. The first degradation sequence, PEST, is a forty-amino acid sequence isolated from the C-terminal region of mouse ornithine decarboxylase (2). The second consists of CL1, PEST and one mRNA degradation sequence, ARE (AU-rich element). CL1 has been shown to increase protein degradation (3). PEST and CL1-PEST have been designed to reduce the number of mammalian consensus transcription factor binding sites. ARE is a synthetic consensus sequence based on the 3´-untranslated region of *Herpesvirus saimiri* small nuclear RNA. These ARE are present in the 3´-untranslated regions of many early response genes where their presence has been shown to promote mRNA degradation.

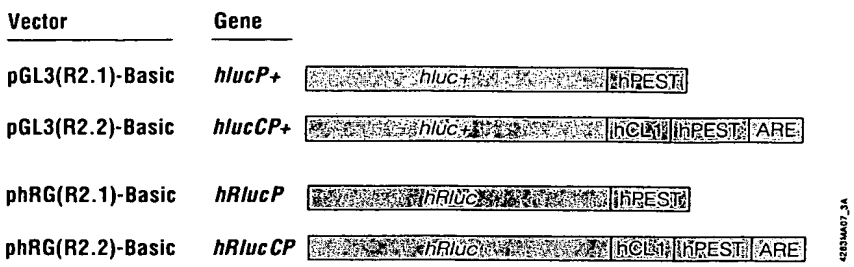


Figure 1. Diagram of the Rapid Response™ Reporter Genes.

The resulting synthetic versions of these degradation sequences are referred to as hPEST and hCL1-hPEST-ARE. The pGL3(R2.1)-Basic and the phRG(R2.1)-Basic Vectors contain the *hlucP+* (firefly) and *hRlucP* (*Renilla*) luciferase genes, respectively, both of which contain the hPEST degradation sequence. The pGL3(R2.2)-Basic and the phRG(R2.2)-Basic Vectors contain the *hlucCP+* (firefly) and the *hRlucCP* (*Renilla*) genes, respectively, and both genes contain the hCL1-hPEST-ARE degradation sequence (Figure 1). The vector backbone used for the four Rapid Response™ Vectors is the pGL3-Basic Vector (Cat.# E1751).

B. Characteristics of the Rapid Response™ Luciferase Proteins

Table 1 shows the reduction in reporter half-life of the luciferase proteins expressed from the Rapid Response™ Vectors. The half-lives of the firefly and *Renilla* Rapid Response™ Vectors have been reduced by more than 60% when compared to the parent synthetic firefly and *Renilla* luciferase proteins.

Because of this reduction in half-life, there is a corresponding reduction in time to achieve maximum-fold induction for all of the Rapid Response™ Reporter proteins (Table 1 and Figure 2). For firefly luciferase, the time to reach maximum fold induction for the control synthetic firefly luciferase gene (*hluc+*) is 6 hours. The firefly-based Rapid Response™ Reporters, hlucP+ and hlucCP+, reach maximum-fold induction in 3.0 and 1.5 hours, respectively. For the *Renilla*-based Rapid Response™ constructs, hRlucP and hRlucCP+, the time to achieve maximum-fold induction compared to the control protein is also reduced. The hRluc control reaches maximum-fold induction at 8 hours, while the hRlucCP and hRlucP reach maximum-fold induction at 3.0 and 4.5 hours, respectively.

Promega

Note: Varying assay conditions will affect assay results.

Table 1. Characteristics of the Rapid Response™ Luciferase Reporter Proteins.

Vector	pGL3-Control	pGL3-hluc+[2]	pGL3 (R2.1)-Basic	pGL3 (R2.2)-Basic	phRG-B	phRG (R2.1)-Basic	phRG (R2.2)-Basic
Expressed Reporter Protein	luc+	hluc+	hlucP+	hlucCP+	hRluc	hRlucP	hRlucCP
Reporter Protein Half-Life (hours)[1]	3.4	3.2	1.0	0.6	3.0	1.0	0.4
Time to Achieve Maximum Induction (hours)	6.0	6.0	3.0	1.5	8.0	4.5	3.0

[1]Rapid Response™ Reporters and control synthetic luciferase genes were cloned into the pGL3-Control Vector[d,g] and transfected into CHO cells. Twenty-four hours later, cycloheximide (100µg/ml final concentration) was added to each well. At specific time points, cells were collected and the relative light units determined using either the Luciferase Assay System[b,h] or the *Renilla* Luciferase Assay System[a,b]. The firefly luc+ and hluc+ reporter proteins are 99.8% identical, with only one amino acid difference.

[2]pGL3-hluc+ is not commercially available.

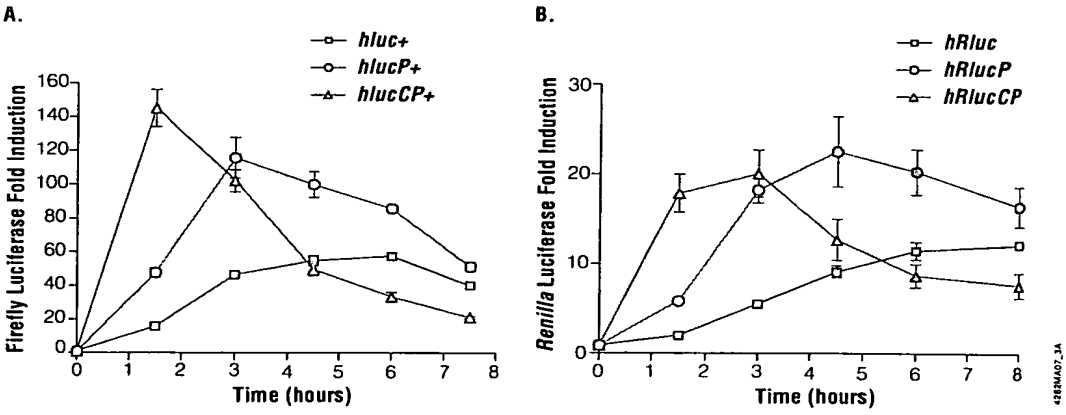


Figure 2. Time to achieve maximum-fold induction. To determine maximum-fold induction a DNA segment containing multiple CREs (cAMP Response Element) was cloned into each of the four Rapid Response™ Reporter Vectors and the two control vectors containing *hluc+* and *hRluc*. The CRE-containing vectors were then transiently transfected into d293 cells (a subpopulation of HEK 293 cells). Twenty-four hours later 100µM of RO (RO-20-1724) and 1µM of ISO (isoproterenol hydrochloride) were added to the transiently-transfected cells to induce reporter gene expression. RO alone (100µM) was added to a subset of the wells to serve as a noninduced control. Cells were harvested, lysed and assayed with either the Luciferase Assay System (Cat.# E1500; **Panel A**) or the *Renilla* Luciferase Assay System (Cat.# E2810; **Panel B**). Fold induction was calculated by dividing the relative light units obtained from the induced wells by the relative light units obtained from noninduced wells. Due to a large genetic difference between *hluc+* and *luc+* (>30%), *hluc+* was selected as the appropriate control.

The data in Figure 2 further detail the responsiveness of the Rapid Response™ Reporter Vectors. In Panel A at 1.5 hours the hluc+ control reaches ~16-fold induction. However, hlucCP+ and hlucP+ reach 145- and 48-fold induction, respectively, in the same 1.5 hours. Thus the percentage increase in reporter response (see equation below) for hlucCP+ and hlucP+ has increased 806% and 200%, respectively. In Panel B at 1.5 hours the hRluc control displays a 2-fold increase while hRlucCP displays a 18-fold increase over the same time frame; thus hRlucCP displays an 800% increase in reporter response. For the hRlucP a 6-fold increase is demonstrate for the 1.5 hour time point or a 200% increase in reporter response is demonstrated.

Reporter response is the percent change in the fold induction for a Rapid Response™ Reporter Vector over its respective control for the same time interval.

Reporter response =

$$\frac{[(\text{fold induction for hlucCP+}) - (\text{fold induction for hluc+})]}{(\text{fold induction for hluc+})} \times 100$$

$$\text{Reporter response for hlucCP+} = \frac{[145 - 16]}{16} \times 100 = 806\%$$

C. Protein Degradation Rate and Signal Intensity

Destabilized luciferase proteins are not accumulated in the cell to the same extent as their wildtype counterparts. As a result, destabilized reporter proteins typically generate lower signal intensities. The hlucP+ protein displays 51% (in CHO cells) and 203% (in NIH3T3 cells) of the relative light units obtained using the firefly luciferase gene (*luc+*) (Figure 3). Firefly luciferase hlucCP+ protein displays approximately 2% (in both CHO and NIH3T3 cells) of the relative light units of the pGL3-Control firefly *luc+* control (Figure 3, Panel A).

In Figure 3, Panel B, the reporter protein hRlucP displays 18.6% (in CHO cells) and 3.2% (in NIH3T3 cells) of the relative light units of the hRluc control. As with the hlucCP+ protein, the *Renilla* hRlucCP reporter protein displays the lowest signal intensity, having 10.3% (in CHO cells) and 0.6% (in NIH3T3 cells) of the relative light units of the hRluc control. Note that the relative light units obtained using the Rapid Response™ Reporter Vectors can vary depending on the mammalian cell line used.

Relative light units obtained using the Rapid Response™ Reporters can vary depending on the mammalian cell line used.

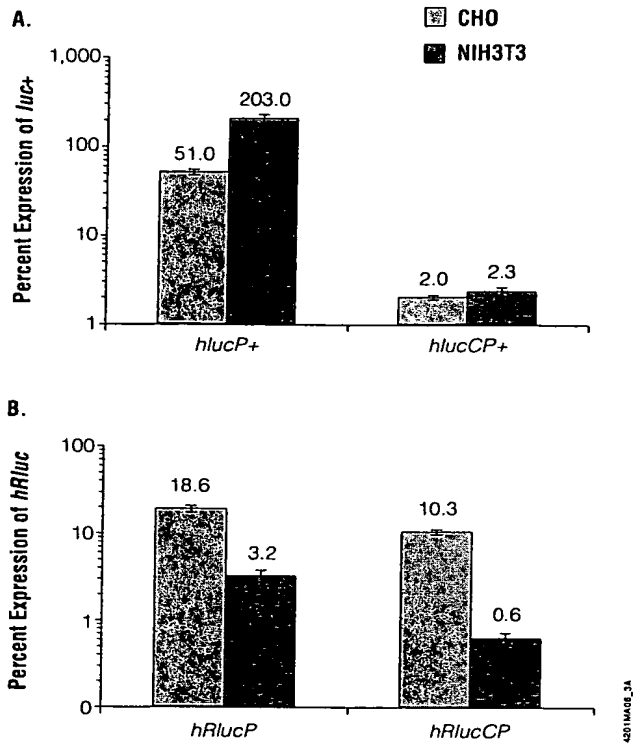


Figure 3. Expression of Rapid Response™ luciferase genes in CHO and NIH3T3 cells. The *luc+* gene present in pGL3-Control Vector (Cat.# E1741) was replaced with genes encoding either the hlucP+, hlucCP+, hRluc, hRlucP, or hRlucCP proteins. Thus the only difference among the various vectors is the reporter gene. The resulting vectors and the pGL3-Control Vector were cotransfected with a second reporter (transfection control) into either CHO or NIH3T3 cells. The transfection controls for **Panels A** and **B** were phRL-TK (Cat.# E6291) and pGL3-Control (Cat.# E1741) Vectors, respectively. Twenty-four hours post-transfection the cells were harvested with Passive Lysis Buffer (Cat.# E1941), and relative light units were determined using the Dual-Luciferase® Assay System[d,h,i] (Cat.# E1910). The relative light units were normalized to the transfection control. The effects of the destabilization sequences on the accumulation of luciferase enzyme are shown as percent of the control. **Panel A.** Percent expression of hlucP+ and hlucCP+ versus luc+ (pGL3-Control Vector) in CHO and NIH3T3 cell transfections. **Panel B.** Percent expression of hRlucP and hRlucCP versus hRluc in CHO and NIH3T3 cell transfections.

IV. Rapid Response™ Reporter Vector Maps

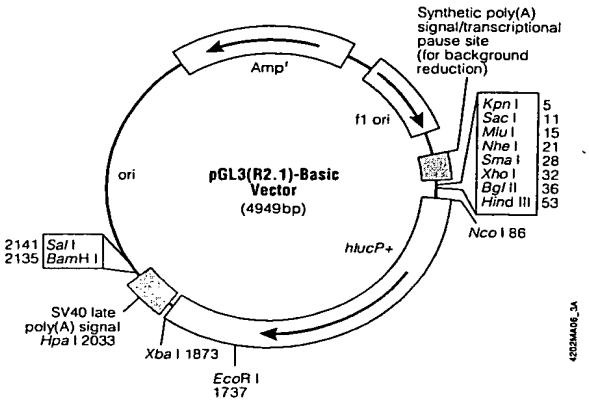


Figure 4. pGL3(R2.1)-Basic Vector circle map.

pGL3(R2.1)-Basic Vector Sequence Reference Points:

Multiple cloning region	1–58
hlucP+ reporter gene (synthetic firefly luciferase; includes hPEST)	88–1863
SV40 late poly(A) region	1903–2124
Reporter Vector Primer 4 (RVPrimer4) binding site	2192–2211
*Col*E1-derived origin of replication	2449
β-lactamase (Amp^r) coding region	3211–4071
f1 origin of replication*	4203–4658
Upstream synthetic poly(A) region	4789–4942
Reporter Vector Primer 3 (RVPrimer3) binding site	4891–4910

*f1 origin of replication verified by sequence only

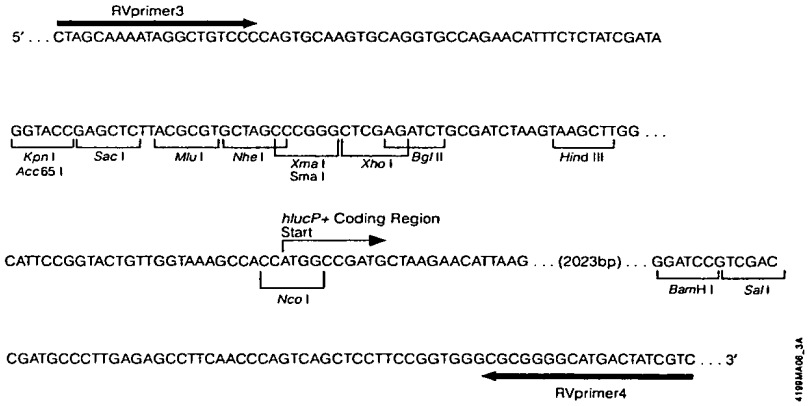


Figure 5. pGL3(R2.1)-Basic Vector synthetic firefly luciferase flanking regions including the multiple cloning region.

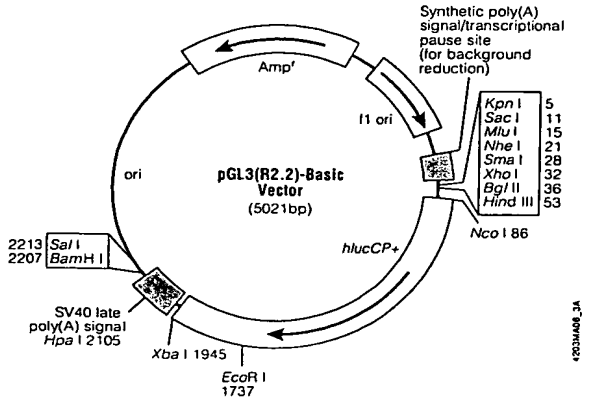


Figure 6. pGL3(R2.2)-Basic Vector circle map.

pGL3(R2.2)-Basic Vector Sequence Reference Points:

Multiple cloning region	1–58
hlucCP+ reporter gene (synthetic firefly luciferase; includes hCL1, hPEST and ARE)	88–1944
SV40 late poly(A) region	1975–2196
Reporter Vector Primer 4 (RVPrimer4) binding site	2264–2283
*Col*E1-derived origin of replication	2521
β-lactamase (Amp^r) coding region	3283–4143
f1 origin of replication*	4275–4730
Upstream synthetic poly(A) region	4861–5014
Reporter Vector Primer 3 (RVPrimer3) binding site	4963–4982

*f1 origin of replication verified by sequence only

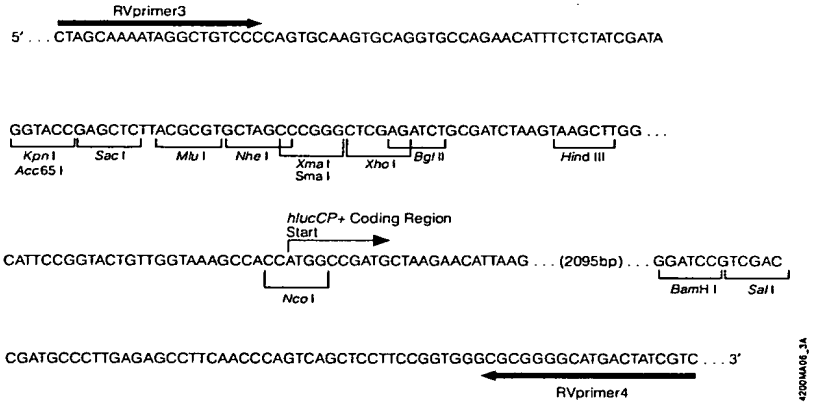


Figure 7. pGL3(R2.2)-Basic Vector synthetic firefly luciferase flanking regions including the multiple cloning region.

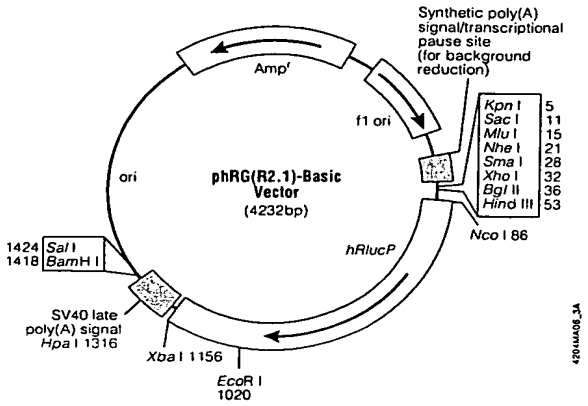


Figure 8. phRG(R2.1)-Basic Vector circle map.

phRG(R2.1)-Basic Vector Sequence Reference Points:

Multiple cloning region	1–58
hRlucP reporter gene (synthetic *Renilla* luciferase; includes hPEST)	88–1146
SV40 late poly(A) region	1186–1407
Reporter Vector Primer 4 (RVPrimer4) binding site	1475–1494
*Col*E1-derived origin of replication	1732
β-lactamase (Amp^r) coding region	2494–3354
f1 origin of replication*	3486–3941
Upstream synthetic poly(A) region	4072–4225
Reporter Vector Primer 3 (RVPrimer3) binding site	4174–4193

*f1 origin of replication verified by sequence only

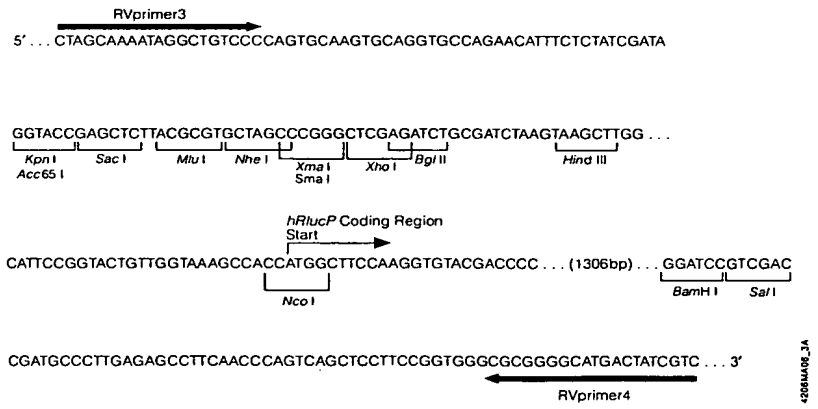


Figure 9. phRG(R2.1)-Basic Vector synthetic *Renilla* luciferase flanking regions including the multiple cloning region.

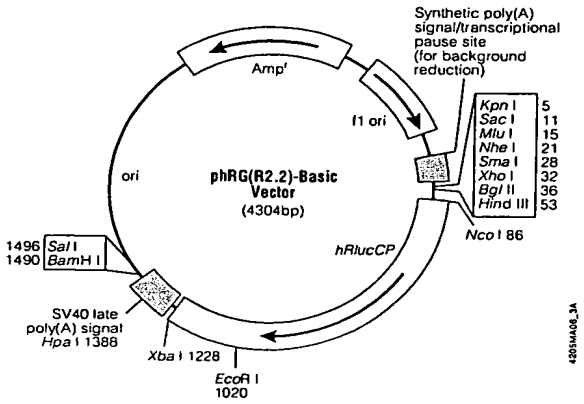


Figure 10. phRG(R2.2)-Basic Vector circle map.

phRG(R2.2)-Basic Vector Sequence Reference Points:

Multiple cloning region	1–58
hRlucCP reporter gene (synthetic *Renilla* luciferase, including hCL1, hPEST and ARE)	88–1227
SV40 late poly(A) region	1258–1479
Reporter Vector Primer 4 (RVPrimer4) binding site	1547–1566
*Col*E1-derived origin of replication	1804
β-lactamase (Amp^r) coding region	2566–3426
f1 origin of replication*	3558–4013
Upstream synthetic poly(A) region	4144–4297
Reporter Vector Primer 3 (RVPrimer3) binding site	4246–4265

*f1 origin of replication verified by sequence only

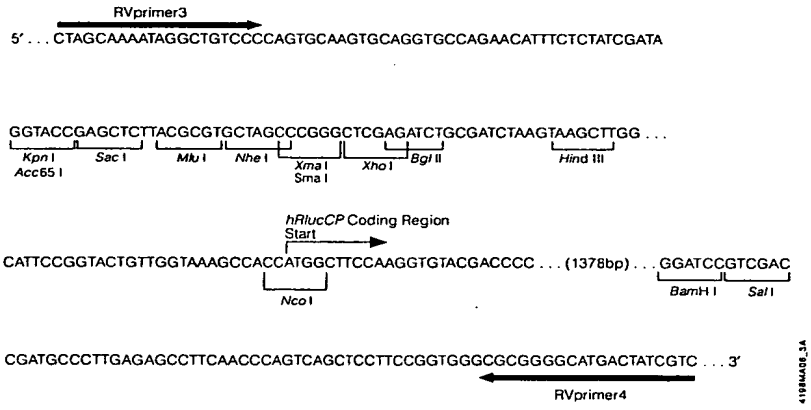


Figure 11. phRG(R2.2)-Basic Vector synthetic *Renilla* luciferase flanking regions including the multiple cloning region.

V. References

1. Schimke R.T. (1973) Control of enzyme levels in mammalian tissues. *Adv. Enzymol. Related Areas Mol. Biol.* **37**, 135–87.
2. Li, X. *et al.* (1998) Generation of destabilized green fluorescent protein as a transcription reporter. *J. Biol. Chem.* **273**, 34970–5.
3. Gilon, T., Chomsky, O. and Kulka, R.G. (1998) Degradation signals for ubiquitin system proteolysis in *Saccharomyces cerevisiae*. *EMBO. J.* **17**, 2759–66.
4. Fan, X.C., Myer, V.E. and Steitz, J.A. (1997) AU-rich elements target small nuclear RNAs as well as mRNAs for rapid degradation. *Genes Dev.* **11**, 2557–68.
5. Lagnado, C.A., Brown, C.Y. and Goodall, G.J. (1994) AUUUA is not sufficient to promote poly(A) shortening and degradation of an mRNA: The functional sequence within AU-rich elements may be UUAUUUA (U/A)(U/A). *Mol. Cell. Biol.* **14**, 7984–95.
6. Zubiaga, A.M., Belagco, J.G. and Greenberg, M.E. (1995) The nonamer UUAUU-UAUU is the key AU-rich sequence motif that mediates mRNA degradation. *Mol. Cell. Biol.* **15**, 2219–30.

VI. Related Products

Firefly and *Renilla* Luciferase Assay Systems

Product	Size	Cat.#
Dual-Glo™ Luciferase Assay System[b,i,j]	10ml	E2920
	100ml	E2940
	10 x 100ml	E2980
Bright-Glo™ Luciferase Assay System[b,i,j]	10ml	E2610
	100ml	E2620
	10 x 100ml	E2650
Steady-Glo® Luciferase Assay System[b,i,j]	10ml	E2510
	100ml	E2520
	10 x 100ml	E2550
Luciferase Assay System[b,h]	100 assays	E1500
Luciferase 1000 Assay System[b,h]	1,000 assays	E4550
Luciferase Assay System with Reporter Lysis Buffer[b,h]	100 assays	E4030
Dual-Luciferase® Reporter Assay System[b,i,j]	100 assays	E1910
Dual-Luciferase® Reporter 1,000 Assay System[b,i,j]	1,000 assays	E1980
Renilla Luciferase Assay System[a,b]	100 assays	E2810
	1,000 assays	E2820

Lysis Buffer

Product	Size	Cat.#
Passive Lysis 5X Buffer	30ml	E1941

Promega

VI. Related Products (continued)

Synthetic *Renilla* Luciferase Vectors

Product	Size	Cat.#
phRL-null Vector[a,b,c,h]	20µg	E6231
phRL-TK Vector[a,b,c,h]	20µg	E6241
phRL-TK(Int–) Vector[a,b,c,h]	20µg	E6251
phRL-SV40 Vector[a,b,c,h]	20µg	E6261
phRL-CMV Vector[a,b,c,h,k]	20µg	E6271
phRG-B Vector[a,b,c,h]	20µg	E6281
phRG-TK Vector[a,b,c,h]	20µg	E6291

Firefly Luciferase Vectors

Product	Size	Cat.#
pGL3-Control Vector[d,g]	20µg	E1741
pGL3-Basic Vector[d,g]	20µg	E1751
pGL3-Promoter Vector[d,g]	20µg	E1761
pGL3-Enhancer Vector[d,g]	20µg	E1771

VII. Appendix

A. pGL3(R2.1)-Basic Vector Restriction Enzyme Tables

The following restriction enzyme tables were constructed using DNASTAR® sequence analysis software. Please note that we have not verified this information by restriction digestion with each enzyme listed. The location given specifies the 3′-end of the cut DNA (the base to the left of the cut site). For more information on the cut sites of these enzymes, or if you identify a discrepancy, please contact your local Promega Branch or Distributor. In the U.S., contact Promega Technical Services at 800-356-9526. Vector sequences are also available in the GenBank® database (GenBank®/EMBL Accession Number AY487821) and online at: **www.promega.com/vectors/**

Table 2. Restriction Enzymes That Cut the pGL3(R2.1)-Basic Vector Between 1 and 5 Times.

Enzyme	# of Sites	Location
Acc I	2	688, 2142
Acc65 I	1	1
Afl III	2	15, 2391
Alw26 I	4	1111,1409, 3345, 4121
Alw44 I	2	2705, 3951
*Alw*N I	1	2807
Apa I	1	118
Ava I	3	26, 32, 1756
Ava II	4	1209,1392, 3422, 3644
Bal I	2	1069, 1594
BamH I	1	2135
Ban II	4	11, 33, 118, 4362
Bbe I	3	371, 1037, 1169
Bbs I	1	2220
Bcl I	3	668, 1409, 1703
Bgl I	5	88, 1492, 1667, 3404, 4672
Bgl II	1	36
Bsa I	1	3345
*Bsa*A I	2	1290, 4433
*Bsa*B I	3	535, 1702, 2134
BsaM I	3	60, 1954, 2047
Bsm I	3	60, 1954, 2047
*Bsp*120 I	1	114
*Bsp*H I	3	671, 3111, 4119
*Bsp*M I	2	1019, 4912
*Bsr*G I	1	578
*Bss*S I	3	1015, 2564, 3948
BstX I	1	1206
BstZ I	3	1886, 1890, 4782
Bsu36 I	2	701, 1301
Cla I	3	2128, 4840, 4944
Dra I	4	2094, 3150, 3169, 3861
Dra II	4	114, 115, 480, 1659
Dra III	1	4436
Drd I	3	1876, 2499, 4480
Eag I	3	1886, 1890, 4782
Ear I	5	172, 283, 2275, 4079, 4717
Ecl HK I	1	3284
Eco47 III	2	1075, 2267
Eco52 I	3	1886, 1890, 4782
*Eco*81 I	2	701, 1301

Enzyme	# of Sites	Location
EcoICR I	1	9
*Eco*N I	3	277, 700, 1705
EcoR I	1	1737
Ehe I	3	369, 1035, 1167
Fse I	2	1499, 1892
Fsp I	3	910, 3506, 4679
Hinc II	2	2033, 2143
Hind II	2	2033, 2143
Hind III	1	53
Hpa I	1	2033
Kas I	3	367, 1033, 1165
Kpn I(i)	1	5
Mlu I	1	15
MspA1 I	5	151, 162, 2733, 2978, 3919
Nar I	3	368, 1034, 1166
Nci I	5	27, 28, 2771, 3467, 3818
Nco I	1	86
Nhe I	1	21
Not I	1	4782
Nsp I	3	579, 834, 2395
*Pae*R7 I	1	32
*Psh*A I	1	2206
*Psp*A I	1	26
Pst I	3	347, 488, 1466
Pvu I	2	3654, 4700
Pvu II	1	162
Sac I	1	11
Sal I	1	2141
Sca I	3	253, 3764, 4847
Sin I	4	1209, 1392, 3422, 3644
Sma I	1	28
Srf I	1	28
Ssp I	3	4088, 4641, 4756
Sty I	3	86, 1199, 1655
Tfi I	2	2366, 4837
Vsp I	1	3456
Xba I	1	1873
Xho I	1	32
Xma I	1	26
Xmn I	1	3883

Note: The enzymes listed in boldface type are available from Promega.

Promega

Table 3. Restriction Enzymes That Do Not Cut the pGL3(R2.1)-Basic Vector.

Aat II	**_Bbu_ I**	**_Csp_45 I**	*Pfl*M I	**_Sac_ II**	*Sse*8387 I
_Acc_B7 I	*Blp* I	*Eco*72 I	*Pin*A I	**_Sfi_ I**	**_Stu_ I**
Acc III	*Bpu*1102 I	**_Eco_R V**	*Pme* I	**_Sgf_ I**(I)	*Swa* I
Afl II	**_Bss_H II**	**I-_Ppo_ I**	*Pml* I	*Sgr*A I	**_Tth_111 I**
Age I	*Bst*1107 I	**_Nde_ I**	*Ppu*10 I	**_Sna_B I**	*Xcm* I
Asc I	**_Bst_98 I**	**_Nru_ I**	*Ppu*M I	**_Spe_ I**	
Avr II	**_Bst_E II**	**_Nsi_ I**	*Psp*5 II	**_Sph_ I**	
*Bbr*P I	**_Csp_ I**	*Pac* I	*Rsr* II	*Spl* I	

Table 4. Restriction Enzymes That Cut the pGL3(R2.1)-Basic Vector 6 or More Times.

Aci I	*Bsr* I	*Dsa* I	*Hph* I	**_Msp_ I**	*Scr*F I
Acy I	**_Bsr_ S I**	*Eae* I	**_Hsp_92 I**	**_Nae_ I**	*Sfa*N I
Alu I	*Bst*71 I	*Fnu*4H I	**_Hsp_92 II**	**_Nde_ II**	**_Taq_ I**
*Asp*H I	**_Bst_O I**	**_Fok_ I**	*Mae* I	**_Ngo_M IV**	**_Tru_9 I**
Ban I	*Bst*U I	**_Hae_ II**	*Mae* II	*Nla* III	**_Xho_ II**
Bbv I	**_Cfo_ I**	**_Hae_ III**	*Mae* III	*Nla* IV	
*Bsa*O I	*Cfr*10 I	*Hga* I	*Mbo* I	*Ple* I	
*Bsa*H I	**_Dde_ I**	**_Hha_ I**	**_Mbo_ II**	**_Rsa_ I**	
*Bsa*J I	**_Dpn_ I**	**_Hin_f I**	*Mnl* I	**_Sau_3A I**	
_Bsp_1286 I	*Dpn* II	**_Hpa_ II**	*Mse* I	*Sau*96 I	

Note: The enzymes listed in boldface type are available from Promega.

B. pGL3(R2.2)-Basic Vector Restriction Enzyme Tables

The following restriction enzyme tables were constructed using DNASTAR® sequence analysis software. Please note that we have not verified this information by restriction digestion with each enzyme listed. The location given specifies the 3′-end of the cut DNA (the base to the left of the cut site). For more information on the cut sites of these enzymes, or if you identify a discrepancy, please contact your local Promega Branch or Distributor. In the U.S., contact Promega Technical Services at 800-356-9526. Vector sequences are also available in the GenBank® database (GenBank®/EMBL Accession Number AY487822) and online at: **www.promega.com/vectors/**

Table 5. Restriction Enzymes That Cut the pGL3(R2.2)-Basic Vector Between 1 and 5 Times.

Enzyme	# of Sites	Location
Acc I	2	688, 2214
*Acc*65 I	1	1
Afl II	1	1767
Afl III	2	15, 2463
Alw26 I	4	1111, 1409, 3417, 4193
Alw44 I	2	2777, 4023
*Alw*N I	1	2879
Apa I	1	118
Ava I	3	26, 32, 1810
Ava II	4	1209, 1392, 3494, 3716
Bal I	2	1069, 1594
BamH I	1	2207
Ban II	4	11, 33, 118, 4434
Bbe I	3	371, 1037, 1169
Bbs I	1	2292
Bcl I	3	668, 1409, 1703
Bgl I	5	88,1492,1667, 3476, 4744
Bgl II	1	36
Bsa I	1	3417
*Bsa*A I	2	1290, 4505
*Bsa*B I	3	535, 1702, 2206
BsaM I	3	60, 2026, 2119
Bsm I	3	60, 2026, 2119
*Bsp*120 I	1	114
*Bsp*H I	3	671, 3183, 4191
*Bsp*M I	2	1019, 4984
*Bsr*G I	1	578
*Bss*S I	3	1015, 2636, 4020
Bst98 I	1	1767
BstX I	1	1206
BstZ I	3	1958, 1962, 4854
Bsu36 I	2	701, 1301
Cla I	3	2200, 4912, 5016
Dra I	4	2166, 3222, 3241, 3933
Dra II	4	114, 115, 480, 1659
Dra III	2	1779, 4508
Drd I	2	2571, 4552
Eag I	3	1958, 1962, 4854
Ear I	5	172, 283, 2347, 4151, 4789
EclHK I	1	3356
Eco47 III	2	1075, 2339
Eco52 I	3	1958, 1962, 4854
*Eco*81 I	2	701, 1301
EcoICR I	1	9
*Eco*N I	3	277, 700, 1705
EcoR I	1	1737
Ehe I	3	369, 1035, 1167
Fse I	2	1499, 1964
Fsp I	3	910, 3578, 4751
Hinc II	2	2105, 2215
Hind II	2	2105, 2215
Hind III	1	53
Hpa I	1	2105
Kas I	3	367, 1033, 1165
Kpn I	1	5
Mlu I	1	15
MspA1 I	5	151, 162, 2805, 3050, 3991
Nar I	3	368, 1034, 1166
Nci I	5	27, 28, 2843, 3539, 3890
Nco I	1	86
Nhe I	1	21
Not I	1	4854
Nsp I	3	579, 834, 2467
*Pae*R7 I	1	32
*Psh*A I	1	2278
*Psp*A I	1	26
Pst I	3	347, 488, 1466
Pvu I	2	3726, 4772
Pvu II	1	162
Sac I	1	11
Sal I	1	2213
Sca I	3	253, 3836, 4919
Sin I	4	1209, 1392, 3494, 3716
Sma I	1	28
Srf I	1	28
Ssp I	3	4160, 4713, 4828
Sty I	3	86, 1199, 1655
Tfi I	2	2438, 4909
Vsp I	1	3528
Xba I	1	1945
Xho I	1	32
Xma I	1	26
Xmn I	2	1756, 3955

Note: The enzymes listed in boldface type are available from Promega.

Promega

Table 6. Restriction Enzymes That Do Not Cut the pGL3(R2.2)-Basic Vector.

***Aat* II**	*Blp* I	***Eco*R V**	*Pme* I	***Sgf* I**	*Swa* I
***Acc*B7 I**	*Bpu*1102 I	**I-*Ppo* I**	*Pml* I	*Sgr*A I	***Tth*111 I**
***Acc* III**	***Bss*H II**	***Nde* I**	*Ppu*10 I	***Sna*B I**	***Xcm* I**
***Age* I**	*Bst*1107 I	***Nru* I**	*Ppu*M I	***Spe* I**	
Asc I	***Bst*E II**	***Nsi* I**	*Psp*5 II	***Sph* I**	
Avr II	***Csp* I**	*Pac* I	*Rsr* II	*Spl* I	
*Bbr*P I	***Csp*45 I**	*Pfl*M I	***Sac* II**	*Sse*8387 I	
***Bbu* I**	*Eco*72 I	*Pin*A I	***Sfi* I**	***Stu* I**	

Table 7. Restriction Enzymes That Cut the pGL3(R2.2)-Basic Vector 6 or More Times.

Aci I	*Bsr* I	*Dsa* I	*Hph* I	*Msp* I	*Scr*F I
Acy I	***Bsr*S I**	*Eae* I	***Hsp*92 I**	***Nae* I**	*Sfa*N I
***Alu* I**	*Bst*71 I	*Fnu*4H I	***Hsp*92 II**	***Nde* II**	***Taq* I**
*Asp*H I	***Bst*O I**	***Fok* I**	*Mae* I	***Ngo*M IV**	***Tru*9 I**
***Ban* I**	*Bst*U I	***Hae* II**	*Mae* II	*Nla* III	***Xho* II**
Bbv I	***Cfo* I**	***Hae* III**	*Mae* III	*Nla* IV	
*Bsa*O I	*Cfr*10 I	*Hga* I	*Mbo* I	*Ple* I	
*Bsa*H I	***Dde* I**	***Hha* I**	***Mbo* II**	***Rsa* I**	
*Bsa*J I	***Dpn* I**	***Hin*f I**	*Mnl* I	***Sau*3A I**	
***Bsp*1286 I**	*Dpn* II	***Hpa* II**	*Mse* I	*Sau*96 I	

Note: The enzymes listed in boldface type are available from Promega.

C. phRG(R2.1)-Basic Vector Restriction Enzyme Tables

The following restriction enzyme tables were constructed using DNASTAR® sequence analysis software. Please note that we have not verified this information by restriction digestion with each enzyme listed. The location given specifies the 3´-end of the cut DNA (the base to the left of the cut site). For more information on the cut sites of these enzymes, or if you identify a discrepancy, please contact your local Promega Branch or Distributor. In the U.S., contact Promega Technical Services at 800-356-9526. Vector sequences are also available in the GenBank® database (GenBank®/EMBL Accession Number AY487823) and online at: **www.promega.com/vectors/**

Table 8. Restriction Enzymes That Cut the phRG(R2.1)-Basic Vector Between 1 and 5 Times.

Enzyme	# of Sites	Location
***Aat* II**	1	785
***Acc* I**	1	1425
***Acc*65 I**	1	1
Acy I	2	782, 3104
Afl III	2	15, 1674
***Alw*26 I**	3	628, 2628, 3404
***Alw*44 I**	2	1988, 3234
*Alw*N I	1	2090
*Asp*H I	5	11, 485, 1992, 3153, 3238
***Ava* I**	4	26, 32, 109, 1039
***Ava* II**	2	2705, 2927
***Bam*H I**	1	1418
***Ban* II**	5	11, 33, 153, 293, 3645
Bbs I	1	1503
***Bcl* I**	2	128, 581
***Bgl* I**	3	88, 2687, 3955
***Bgl* II**	1	36
Bsa I	2	628, 2628
*Bsa*A I	1	3716
*Bsa*B I	2	847, 1417
*Bsa*H I	2	782, 3104
***Bsa*M I**	3	60, 1237, 1330
Bsm I	3	60, 1237, 1330
*Bsp*H I	2	2394, 3402
*Bsp*M I	1	4195
*Bss*S I	2	1847, 3231
***Bst*Z I**	3	1169, 1173, 4065
***Bsu*36 I**	1	674
*Cfr*10 I	5	1060, 1171, 1542, 2647, 3611
***Cla* I**	3	1411, 4123, 4227
***Dra* I**	4	1377, 2433, 2452, 3144
Dra II	1	933
Dra III	2	276, 3719
Drd I	3	1159, 1782, 3763
Dsa I	2	86, 293
Eae I	4	1169, 1173, 2955, 4065
Eag I	3	1169, 1173, 4065
Ear I	4	587, 1558, 3362, 4000
***Ecl*HK I**	1	2567
***Eco*47 III**	1	1550
***Eco*52 I**	3	1169, 1173, 4065
*Eco*81 I	1	674
***Eco*ICR I**	1	9
***Eco*R I**	1	1020
***Eco*R V**	1	573
***Fok* I**	4	295, 2533, 2714, 3001
Fse I	1	1175
Fsp I	2	2789, 3962
***Hae* II**	5	1130, 1552, 1922, 3561, 3569
Hga I	5	964, 1785, 2363, 3093, 3494
***Hinc* II**	2	1316, 1426
Hind II	2	1316, 1426
***Hind* III**	1	53
***Hpa* I**	1	1316
***Hsp*92 I**	2	782, 3104
***Kpn* I**	1	5
***Mlu* I**	1	15
***Msp*A1 I**	3	2016, 2261, 3202
***Nae* I**	4	1062, 1173, 1544, 3613
***Nci* I**	5	27, 28, 2054, 2750, 3101
***Nco* I**	1	86
***Ngo*M IV**	4	1060, 1171, 1542, 3611
***Nhe* I**	1	21
***Not* I**	1	4065
***Nru* I**	1	749
***Nsi* I**	1	307
Nsp I	1	1678
*Pae*R7 I	1	32
*Ppu*10 I	1	303
*Psh*A I	1	1489
*Psp*A I	1	26
***Pvu* I**	2	2937, 3983
***Sac* I**	1	11
***Sal* I**	1	1424
***Sca* I**	2	3047, 4130
***Sin* I**	2	2705, 2927
***Sma* I**	1	28
Srf I	1	28
***Ssp* I**	3	3371, 3924, 4039
***Sty* I**	2	86, 95
Tfi I	3	199, 1649, 4120
***Tth*111 I**	1	784
***Vsp* I**	1	2739
***Xba* I**	1	1156
***Xho* I**	1	32
***Xma* I**	1	26
***Xmn* I**	2	622, 3166

Note: The enzymes listed in boldface type are available from Promega.

Promega

Table 9. Restriction Enzymes That Do Not Cut the phRG(R2.1)-Basic Vector.

***Acc* B7 I**	*Bbr*P I	***Bst*E II**	***Nar* I**	***Pst* I**	***Sph* I**
***Acc* III**	***Bbu* I**	***Bst*X I**	***Nde* I**	***Pvu* II**	*Spl* I
Afl II	*Blp* I	***Csp* I**	*Pac* I	*Rsr* II	*Sse*8387 I
***Age* I**	*Bpu*1102 I	***Csp*45 I**	*Pfl*M I	***Sac* II**	***Stu* I**
***Apa* I**	*Bsp*120 I	*Eco*72 I	*Pin*A I	***Sfi* I**	*Swa* I
Asc I	*Bsr*G I	*Eco*N I	*Pme* I	***Sgf* I**	*Xcm* I
Avr II	***Bss*H II**	*Ehe* I	*Pml* I	*Sgr*A I	
***Bal* I**	*Bst*1107 I	**I-*Ppo* I**	*Ppu*M I	***Sna*B I**	
Bbe I	***Bst*98 I**	*Kas* I	*Psp*5 II	***Spe* I**	

Table 10. Restriction Enzymes That Cut the phRG(R2.1)-Basic Vector 6 or More Times.

Aci I	***Bsr*S I**	*Fnu*4H I	*Mae* II	*Nla* III	***Taq* I**
***Alu* I**	*Bsf*71 I	***Hae* III**	*Mae* III	*Nla* IV	***Tru*9 I**
***Ban* I**	***Bst*O I**	***Hha* I**	*Mbo* I	*Ple* I	***Xho* II**
Bbv I	*Bst*U I	***Hin*f I**	***Mbo* II**	***Rsa* I**	
*Bsa*O I	***Cfo* I**	***Hpa* II**	*Mnl* I	***Sau*3A I**	
*Bsa*J I	***Dde* I**	*Hph* I	*Mse* I	*Sau*96 I	
***Bsp*1286 I**	***Dpn* I**	***Hsp*92 II**	***Msp* I**	*Scr*F I	
Bsr I	*Dpn* II	*Mae* I	***Nde* II**	*Sfa*N I	

Note: The enzymes listed in boldface type are available from Promega.

D. phRG(R2.2)-Basic Vector Restriction Enzyme Tables

The following restriction enzyme tables were constructed using DNASTAR® sequence analysis software. Please note that we have not verified this information by restriction digestion with each enzyme listed. The location given specifies the 3′-end of the cut DNA (the base to the left of the cut site). For more information on the cut sites of these enzymes, or if you identify a discrepancy, please contact your local Promega Branch or Distributor. In the U.S., contact Promega Technical Services at 800-356-9526. Vector sequences are also available in the GenBank® database (GenBank®/EMBL Accession Number AY487824) and online at: **www.promega.com/vectors/**

Table 11. Restriction Enzymes That Cut the phRG(R2.2)-Basic Vector Between 1 and 5 Times.

Enzyme	# of Sites	Location
***Aat* II**	1	785
***Acc* I**	1	1497
***Acc*65 I**	1	1
Acy I	2	782, 3176
Afl II	1	1050
Afl III	2	15, 1746
***Alw*26 I**	3	628, 2700, 3476
***Alw*44 I**	2	2060, 3306
*Alw*N I	1	2162
*Asp*H I	5	11, 485, 2064, 3225, 3310
***Ava* I**	4	26, 32, 109, 1093
***Ava* II**	2	2777, 2999
***Bam*H I**	1	1490
***Ban* II**	5	11, 33, 153, 293, 3717
Bbs I	1	1575
***Bcl* I**	2	128, 581
***Bgl* I**	3	88, 2759, 4027
***Bgl* II**	1	36
Bsa I	2	628, 2700
*Bsa*A I	1	3788
*Bsa*B I	2	847, 1489
*Bsa*H I	2	782, 3176
***Bsa*M I**	3	60, 1309, 1402
Bsm I	3	60, 1309, 1402
*Bsp*H I	2	2466, 3474
*Bsp*M I	1	4267
*Bss*S I	2	1919, 3303
***Bst*98 I**	1	1050
***Bst*Z I**	3	1241, 1245, 4137
***Bsu*36 I**	1	674
*Cfr*10 I	5	1114, 1243, 1614, 2719, 3683
***Cla* I**	3	1483, 4195, 4299
***Dra* I**	4	1449, 2505, 2524, 3216
Dra II	1	933
Dra III	3	276, 1062, 3791
Drd I	2	1854, 3835
Dsa I	3	86, 293, 1080
Eae I	4	1241, 1245, 3027, 4137
Eag I	3	1241, 1245, 4137
Ear I	4	587, 1630, 3434, 4072
***Ecl*HK I**	1	2639
***Eco*47 III**	1	1622
***Eco*52 I**	3	1241, 1245, 4137
*Eco*81 I	1	674
***Eco*ICR I**	1	9
***Eco*R I**	1	1020
***Eco*R V**	1	573
***Fok* I**	4	295, 2605, 2786, 3073
Fse I	1	1247
Fsp I	2	2861, 4034
***Hae* II**	5	1184, 1624, 1994, 3633, 3641
Hga I	5	964, 1857, 2435, 3165, 3566
***Hinc* II**	2	1388, 1498
Hind II	2	1388, 1498
***Hind* III**	1	53
***Hpa* I**	1	1388
***Hsp*92 I**	2	782, 3176
***Kpn* I**	1	5
***Mlu* I**	1	15
***Msp*A1 I**	3	2088, 2333, 3274
***Nae* I**	4	1116, 1245, 1616, 3685
***Nci* I**	5	27, 28, 2126, 2822, 3173
***Nco* I**	1	86
***Ngo*M IV**	4	1114, 1243, 1614, 3683
***Nhe* I**	1	21
***Not* I**	1	4137
***Nru* I**	1	749
***Nsi* I**	1	307
Nsp I	1	1750
*Pae*R7 I	1	32
*Ppu*10 I	1	303
*Psh*A I	1	1561
*Psp*A I	1	26
***Pvu* I**	2	3009, 4055
***Sac* I**	1	11
***Sal* I**	1	1496
***Sca* I**	2	3119, 4202
***Sin* I**	2	2777, 2999
***Sma* I**	1	28
Srf I	1	28
***Ssp* I**	3	3443, 3996, 4111
***Sty* I**	2	86, 95
Tfi I	3	199, 1721, 4192
***Tth*111 I**	1	784
***Vsp* I**	1	2811
***Xba* I**	1	1228
***Xho* I**	1	32
***Xma* I**	1	26
***Xmn* I**	3	622, 1039, 3238

Promega

Table 12. Restriction Enzymes That Do Not Cut the phRG(R2.2)-Basic Vector.

AccB7 I	**Bbu** I	**Csp** I	*Pac* I	*Rsr* II	*Sse*8387 I
Acc III	*Blp* I	**Csp**45 I	*Pfl*M I	**Sac** II	**Stu** I
Age I	*Bpu*1102 I	*Eco*72 I	*Pin*A I	**Sfi** I	*Swa* I
Apa I	*Bsp*120 I	*Eco*N I	*Pme* I	**Sgf** I	*Xcm* I
Asc I	*Bsr*G I	*Ehe* I	*Pml* I	*Sgr*A I	
Avr II	**Bss**H II	I-**Ppo** I	*Ppu*M I	**Sna**B I	
Bal I	**Bst**1107 I	*Kas* I	*Psp*5 II	**Spe** I	
Bbe I	**Bst**E II	**Nar** I	**Pst** I	**Sph** I	
*Bbr*P I	**Bst**X I	**Nde** I	**Pvu** II	*Spl* I	

Table 13. Restriction Enzymes That Cut the phRG(R2.2)-Basic Vector 6 or More Times.

Aci I	**Bsr**S I	*Fnu*4H I	*Mae* II	*Nla* III	**Taq** I
Alu I	*Bst*71 I	**Hae** III	*Mae* III	*Nla* IV	**Tru**9 I
Ban I	**Bst**O I	**Hha** I	*Mbo* I	*Ple* I	**Xho** II
Bbv I	*Bst*U I	**Hin**f I	**Mbo** II	**Rsa** I	
*Bsa*O I	**Cfo** I	**Hpa** II	*Mnl* I	**Sau**3A I	
*Bsa*J I	**Dde** I	*Hph* I	*Mse* I	*Sau*96 I	
Bsp1286 I	**Dpn** I	**Hsp**92 II	**Msp** I	*Scr*F I	
Bsr I	*Dpn* II	*Mae* I	**Nde** II	*Sfa*N I	

Note: The enzymes listed in boldface type are available from Promega.

(a)Products may be covered by pending or issued patents.

(b)Certain applications of this product may require licenses from others.

(c)For Nonprofit Organization research use only. Specifically, researchers at Nonprofit Organizations may use this product in their research and they may transfer derivatives of the product to colleagues at other Nonprofit Organizations provided that such colleagues agree in writing to be bound by the terms and conditions of this label license. Researchers may not transfer this product or its derivatives to researchers at other organizations, which are not Nonprofit Organizations, without the express written consent of Promega and without those entities having an express license from Promega for the use of the product. Other than as specifically provided here, an authorized transferee of this product shall have no right to transfer this product or its derivatives to any other person or entity. A Nonprofit Organization performing research using this product for a for-profit organization also shall be considered for-profit. For-profit entities purchasing this product shall have six (6) months to evaluate the product in their research. Thereafter, such for-profit entities shall either contact Promega Corporation and take a license for their continued use of the product or destroy the product and all derivatives thereof. With respect to any therapeutic or diagnostic uses by any entity, please contact Promega Corporation for supply and licensing information.

(d)The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673.

(e)A license (from Promega for research or reagents and from The Regents of the University of California for all other fields) is needed for any commercial sale of nucleic acid contained within or derived from this product.

(f)Licensed under U.S. Pat. Nos. 5,292,658, 5,418,155 and other patents.

(g)U.S. Pat. No. 5,670,356 has been issued to Promega Corporation for a modified luciferase technology.

(h)U.S. Pat. Nos. 5,283,179, 5,641,641, 5,650,289 and 5,814,471, Australian Pat. No. 649289, European Pat. No. 0 553 234 and Japanese Pat. No. 3171595 have been issued to Promega Corporation for a beetle luciferase assay method, which affords greater light output with improved kinetics as compared to the conventional assay. Other patents are pending.

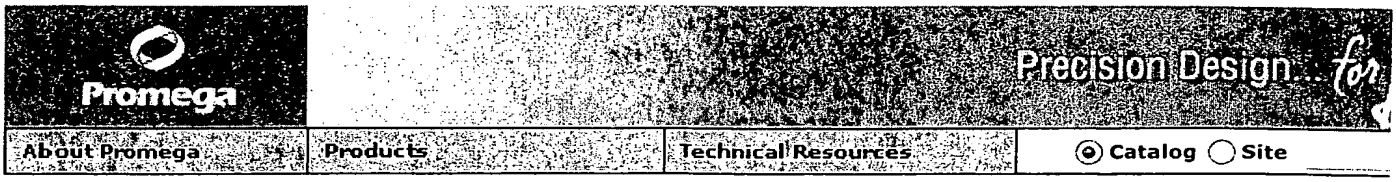
(i)U.S. Pat. No. 5,744,320, Australian Pat. No. 721172 and European Pat. No. 0 833 939 have been issued to Promega Corporation for quenching reagents and assays for enzyme-mediated luminescence. Other patents are pending.

(j)NOTICE TO PURCHASER: LIMITED USE LICENSE

This product is sold under licensing arrangements between Promega Corporation and Invitrogen Corporation. The purchase price of this product includes limited, nontransferable rights under U.S. Pat. Nos. 5,082,784 and 5,192,675 owned by Invitrogen Corporation to use the product only for the internal research purposes of the purchaser. For information on purchasing a license to use the purchased product for purposes other than the internal research of the purchaser, contact the Director of Licensing, Invitrogen Corporation, 1600 Faraday Avenue, Carlsbad, California 92008.

(k)The CMV promoter and its use are covered under U.S. Pat. Nos. 5,168,062 and 5,385,839 owned by the University of Iowa Research Foundation, Iowa City, Iowa, and licensed FOR RESEARCH USE ONLY. Commercial users must obtain a license to these patents directly from the University of Iowa Research Foundation.

(l)U.S. Pat. No. 5,391,487 has been issued to Promega Corporation for Restriction Endonuclease *Sgf* I.



New Products

New Literature

Literature Request

Subscription Request

Unsubscribe

New Products

GENOMICS

Product	Introduced	Protocol
Pronto!™ *Plus* Systems Pronto!™ *Plus* System 1 Pronto!™ *Plus* System 2 Pronto!™ *Plus* System 3 Pronto!™ *Plus* System 4 Pronto!™ *Plus* System 5 Pronto!™ *Plus* System 6	Oct-2003	TM243
MagneSil® Total RNA Mini-Isolation System	Oct-2003	TB328
MagneSil® KF, Genomic System MagneSil® KF, Paramagnetic Particles, 40ml Lysis Buffer, KF, 160ml	Jun-2003	TB322
Wizard® SV 96 PCR Clean-Up System, 100 x 96 preps	May-2003	TB311
MagneSil® Genomic, Fixed Tissue System	May-2003	TB319
PolyATtract® Automated System, 4 x 96 preps	Apr-2003	TB321
RNasin® Plus RNase Inhibitor, 2,500u 10,000u	Apr-2003	
siLentGene™ U6 Cassette RNA Interference System	Mar-2003	TM061

PROTEOMICS

Product	Introduced	Protocol
U6 Hairpin Cloning Systems siSTRIKE™ U6 Hairpin Cloning System—Basic siSTRIKE™ U6 Hairpin Cloning System—Puromycin siSTRIKE™ U6 Hairpin Cloning System—Hygromycin siSTRIKE™ U6 Hairpin Cloning System—Neomycin	Dec-2003	TM246
MagneGST™ Pull-Down System	Dec-2003	TM249
HisLink™ Protein Purification Resin	Dec-2003	TB327
Rapid Response™ Reporter Vectors pGL3(R2.1)-Basic Vector, 20µg pGL3(R2.2)-Basic Vector, 20µg	Dec-2003	TM242

pRG(R2.1)-Basic Vector, 20µg pRG(R2.2)-Basic Vector, 20µg		
FastBreak™ Cell Lysis Reagent 10ml 40ml 100ml	Dec-2003	PIV857
EnduRen™ Live Cell Substrate 0.34mg 3.4mg 34mg	Dec-2003	TM244
MagneGST™ Protein Purification System 40 reactions 200 reactions	Oct-2003	TM240
MagneGST™ Glutathione Particles 4ml 20ml	Oct-2003	TM240
siFECT™ siRNA Transfection Reagent 0.1ml 0.4ml 1.0ml	Oct-2003	TB326
Chroma-Glo™ Luciferase Assay System, 10ml 100ml 10 x 100ml	Jul-2003	TM062
pCBR-Basic Vector pCBR-Control Vector pCBG68-Basic Vector pCBG-Control Vector pCBG99-Basic Vector pCBG99-Control Vector		
T7 RiboMAX™ Express RNAi System	Apr-2003	TB316
siLentGene™ U6 Cassette RNA Interference System	Mar-2003	TM061
Beta-Glo™ Assay System, 10ml 100ml 10 x 100ml	Feb-2003	TM239
Monster Green™ Fluorescent Protein phMGFP Vector, 20µg	Feb-2003	TB320

CELLULAR ANALYSIS

Product	Introduced	Protocol
ProFluor™ Tyrosine Phosphatase Assay 4 plate 8 plate	Jan-2004	TB334
Caspase-Glo™ 8 Assay, 2.5ml 10ml 100ml	Dec-2003	TB332
Caspase-Glo™ 9 Assay, 2.5ml 10ml 100ml	Dec-2003	TB333

ProFluor™ Src-Family Kinase Assay, 4 plate 8 plate	Dec-2003	TB331
p450-Glo™ CYP450 Assays	Jul-2003	TB325
p450-Glo™ CYP1A1 Assay p450-Glo™ CYP1A1 Assay		
p450-Glo™ CYP1B1 Assay p450-Glo™ CYP1B1 Assay		
p450-Glo™ CYP1A2 Assay p450-Glo™ CYP1A2 Assay		
p450-Glo™ CYP2C8 Assay p450-Glo™ CYP2C8 Assay		
p450-Glo™ CYP2C9 Assay p450-Glo™ CYP2C9 Assay		
p450-Glo™ CYP3A4 Assay p450-Glo™ CYP3A4 Assay		
p450-Glo™ CYP3A7 Assay p450-Glo™ CYP3A7 Assay		
CytoTox-ONE™ Homogeneous Membrane Integrity Assay, HTP	Jul-2003	TB306
Also available: CytoTox-ONE™ Homogeneous Membrane Integrity Assay, 200-800 assays 1,000-4,000 assays		
ProFluor™ Ser/Thr Phosphatase Assay, 4 plate 8 plate	Jul-2003	TB324
Caspase-Glo™ 3/7 Assay, 2.5ml 10ml 100ml	Jun-2003	TB323
CellTiter-Blue™ Cell Viability Assay, 20ml 100ml	Jan-2003	TB317

GENETIC IDENTITY

Product	Introduced	Protocol
Y Chromosome Deletion Detection System, Version 2.0	Oct-2003	TM248
PowerPlex® Y System, 200 reactions 50 reactions	Aug-2003	TMD018

Current Applications on New & Existing Promega Products are available on eNotes

ADDITIONAL INFORMATION

Products: For products related to any of the items above, see the individual cat

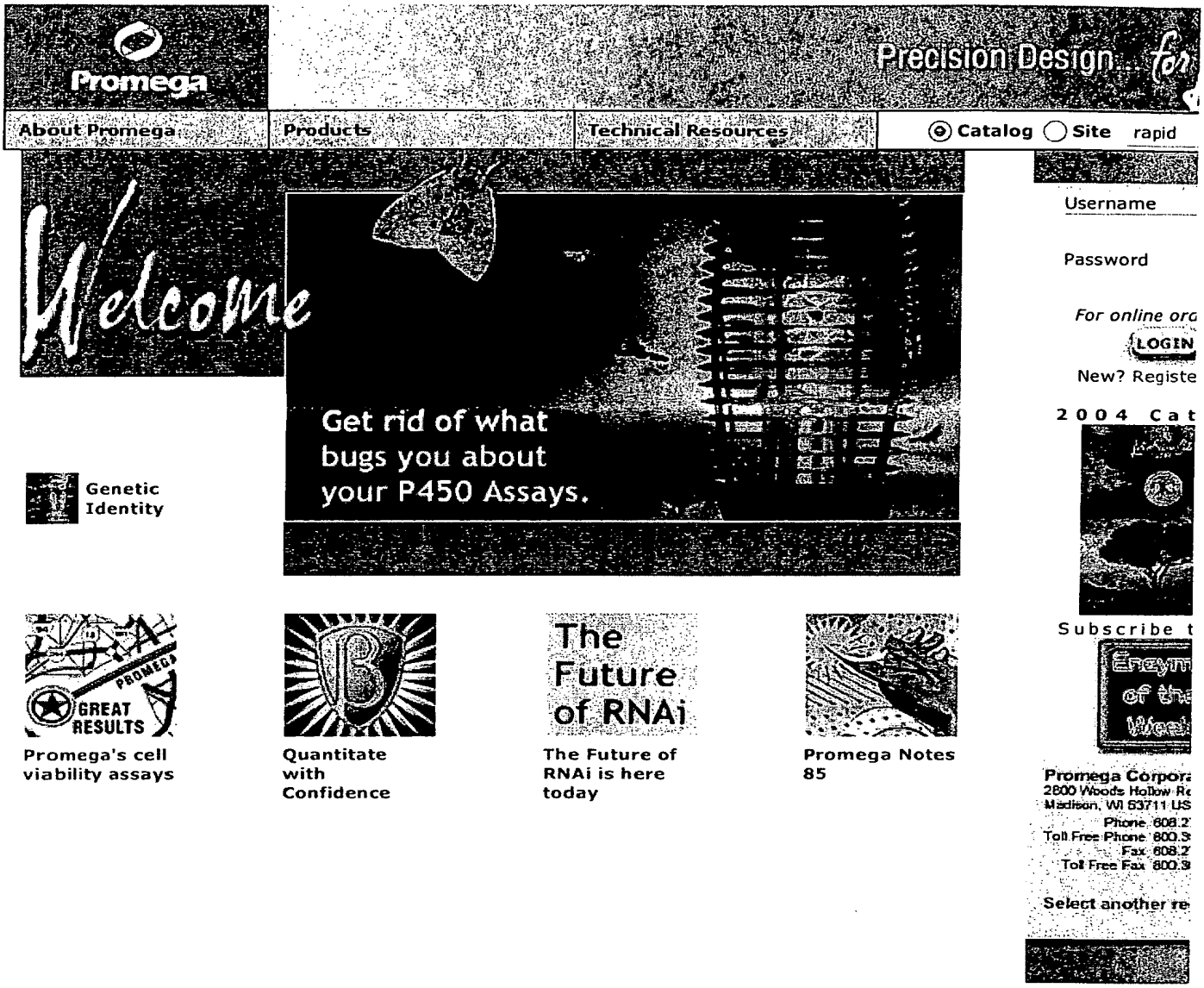
Literature Requests & Subscriptions: To order new product literature, submit a Literature Request or Subs alternatively, call 1-800-356-9526 (within the United States). For cus outside the U.S., contact the nearest Branch Office or Distributor. Ple reference the Literature part number when ordering. Unless specified literature is FREE.

Promotions: Promotional offers are subject to restrictions. Please refer to specific the web page for each promotion.

-top of page-

For problems with this system, contact the Webmaster
For questions regarding orders, contact Customer Service or call 800-356-9526
For technical questions about Promega products, contact Technical Services or call 800-356-9526

Promega Corporation ~ 2800 Woods Hollow Road ~ Madison WI USA 53711 ~ 800-356-9526
Patents, Trademarks and Product Use Information ~ Privacy Policy



For problems with this system, contact the Webmaster
For questions regarding orders, contact Customer Service or call 800-356-9526
For technical questions about Promega products, contact Technical Services or call 800-356-9526

Promega Corporation ~ 2800 Woods Hollow Road ~ Madison WI USA 53711 ~ 800-356-9526
Patents, Trademarks and Product Use Information ~ Privacy Policy

Search Results

You searched for: rapid Total Resul

Rapid Response™ Reporter Vectors

LigaFast™ Rapid DNA Ligation System (M8225)

LigaFast™ Rapid DNA Ligation System (M8221)

ENLITEN® Total ATP Rapid Biocontamination Detection Kit (FF3710)

2X Rapid Ligation Buffer (C6711)

LigaFast™ Rapid DNA Ligation System

ENLITEN® Total ATP Rapid Biocontamination Detection Kit

For problems with this system, contact the Webmaster
For questions regarding orders, contact Customer Service or call 800-356-9526
For technical questions about Promega products, contact Technical Services or call 800-356-9526

Promega Corporation ~ 2800 Woods Hollow Road ~ Madison, WI USA 53711 ~ 800-356-9526
Patents, Trademarks and Product Use Information ~ Privacy Policy

Rapid Response™ Reporter Vectors

Back to Product Groups

Product	Cat.#	Size	Unit	Price	Qty
pGL3(R2.1)-Basic Vector	E6431	20µg	Each	$ 244.00	1
pGL3(R2.2)-Basic Vector	E6441	20µg	Each	$ 244.00	1
phRG(R2.1)-Basic Vector	E6451	20µg	Each	$ 244.00	1
phRG(R2.2)-Basic Vector	E6461	20µg	Each	$ 244.00	1

Component Listing

Description

The Rapid Response™ Reporter Vectors[a,b,c] set a new standard f genes in their ability to respond more quickly and sensitively to ch transcription. The Rapid Response™ Reporters consist of fast- and responding luciferase genes that generate a greater magnitude of in less time, enabling you to significantly decrease incubation time quantitate smaller relative changes in gene expression. Available i synthetic firefly or two synthetic *Renilla* luciferase vector configura Rapid Response™ Reporters are designed for use with Promega's i standard luminescence assay reagents to obtain the most rapid an reporter gene measurements.

Two configurations of the Rapid Response™ Reporters are availabl to select the optimal reporter for your application requirements. D presence of mRNA and protein degradation sequences, the pGL3(F [a,b,c,d] and phRG(R2.2)-Basic[a,b,c,e] Vectors provide the fastest a greatest magnitude of response for firefly and *Renilla* luciferases, respectively. The pGL3(R2.1)-Basic[a,b,c,d] and phRG(R2.1)-Basic[Vectors, which contain protein degradation sequences, also achiev response rates and high fold induction, yet they retain higher total expression. All Rapid Response™ Reporters are provided in a Basic configuration that contains a multiple cloning site to allow for max flexibility in cloning a regulatory element of interest.

Features

- **Up to Fourfold Faster Reporter Response:** Decrease inc time by up to 75% to reduce the risk of secondary effects a improve screening efficiency.
- **Greater Sensitivity:** Achieve a greater magnitude of repo response and quantitate smaller relative changes in gene e
- **Synthetic Gene Design:** Minimize the risk of nonspecific a with reporters that are free of most known consensus sequ transcription factor binding sites. Achieve high expression u codons optimized for mammalian systems.
- **Compatible with Promega's Industry Standard Detect Reagents:** Enjoy rapid, robust and easy-to-use assays in s dual-reporter formats.

Protocol Technical Manual #TM242.

Vector Sequence
pGL3(R2.1)-Basic Vector sequence.
pGL3(R2.2)-Basic Vector sequence.
phRG(R2.1)-Basic Vector sequence.
phRG(R2.2)-Basic Vector sequence.

Storage Conditions Store at –20°C.

GenBank®/EMBL Accession Number pGL3(R2.1)-Basic, AY487821; pGL3(R2.2)-Basic, AY487822; phRG Basic, AY487823; phRG(R2.2)-Basic, AY487824.

Patents/Disclaimers [a]Products may be covered by pending or issued patents.
[b]Certain applications of this product may require licenses from ot
[c]For Nonprofit Organization research use only. Specifically, resea Nonprofit Organizations may use this product in their research anc transfer derivatives of the product to colleagues at other Nonprofit Organizations provided that such colleagues agree in writing to be the terms and conditions of this label license. Researchers may no this product or its derivatives to researchers at other organizations are not Nonprofit Organizations, without the express written conse Promega and without those entities having an express license fron for the use of the product. Other than as specifically provided here authorized transferee of this product shall have no right to transfe product or its derivatives to any other person or entity. A Nonprofi Organization performing research using this product for a for-profi organization also shall be considered for-profit. For-profit entities this product shall have six (6) months to evaluate the product in tl research. Thereafter, such for-profit entities shall either contact Pr Corporation and take a license for their continued use of the produ destroy the product and all derivatives thereof. With respect to an therapeutic or diagnostic uses by any entity, please contact Prome Corporation for supply and licensing information.
[d]The method of recombinant expression of *Coleoptera* luciferase by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673. A license Promega for research reagent products and from The Regents of tl University of California for all other fields) is needed for any comm of nucleic acid contained within or derived from this product.
[e]Licensed under U.S. Pat. Nos. 5,292,658, 5,418,155 and other

Related Products

Renilla Luciferase Assay System
Bright-Glo™ Luciferase Assay System
Dual-Glo™ Luciferase Assay System
Dual-Luciferase® Reporter Assay System
Luciferase Assay Reagent
Luciferase Assay System
Steady-Glo® Luciferase Assay System

For problems with this system, contact the Webmaster
For questions regarding orders, contact Customer Service or call 800-356-9526
For technical questions about Promega products, contact Technical Services or call 800-356-9526

Promega Corporation ~ 2800 Woods Hollow Road ~ Madison, WI USA 53711 ~ 800-356-9526
Patents, Trademarks and Product Use Information ~ Privacy Policy

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
2510548/VPA/sjw		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/AU02/00351	8 March 2002	9 March 2001

Applicant

GENE STREAM PTY LTD et al

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing:

[ ] contained in the international application in written form.

[X] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (See Box II).

4. With regard to the title,

[X] the text is approved as submitted by the applicant.

[ ] the text has been established by this Authority to read as follows:

5. With regard to the abstract,

[X] the text is approved as submitted by the applicant

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the drawings to be published with the abstract is Figure No.

[ ] as suggested by the applicant.

[ ] because the applicant failed to suggest a figure

[ ] because this figure better characterizes the invention

[X] None of the figures

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU02/00351

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: C12N 15/67

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
SEE BELOW

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
SEE BELOW

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN (WPIDS): untranslated region and (destab? or stab?)

DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,Y	WO 99/14346, A (Sequitur, Inc) 25.03.99; see pages 4 and 11-25.	1-10; 22 and 26
X,Y	WO 95/29244, A (Wisconsin Alumni Research Foundation) 02.11.95; see page 5 and claims	1-10, 22 and 26
X,Y	Oncogene 11, pp 2127-2134 (1995) Veyrune et al "c-fos mRNA instability determinants present within both the coding and the 3' non coding region link the degradation of this mRNA to its translation".	1-10, 22 and 26

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 May 2002	17 MAY 2002
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized officer MADHU K. JOGIA Telephone No : (02) 6283 2512

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU02/00351

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,Y	Nucleic Acids Research 27, pp 1164-1673 (1999) Maurer et al "An AU-rich sequence in the 3'-UTR of plasminogen activator inhibitor type 2 (PAI-2) mRNA promotes PAI-2 mRNA decay and provides a binding site for nuclear HuR"	1-10, 22 and 26
X,Y	Molecular and Cellular Biology 21, pp 721-730 (Feb 2001) Dean et al "The 3' Untranslated region of tumor necrosis factor alpha mRNA is a target of the mRNA-stabilizing factor HuR".	1-10, 22 and 26
X	J Biol Chem 275, pp 30248-30255 (2000) Provost et al "Length increase of the human α-globin 3'-untranslated region disrupts stability of the pre-mRNA but not that of the mature mRNA"	1-10, 22 and 26
X	J Biol Chem 275, pp 12963-12969 (2000) Short et al "Structural determinants for post-transcriptional stabilization of lactate dehydrogenase A mRNA by the protein kinase C signal pathway"	1-10, 22 and 26
X	J Biol Chem 273, pp 15749-15757 (1998) Yeilding et al "c-myc mRNA is down-regulated during myogenic differentiation by accelerated decay that depends on translation of regulatory coding elements".	1-10, 22 and 26
X	Molecular and Cellular Biology 17, pp 2698-2707 (1997) Yeilding et al "Coding elements in exons 2 and 3 target c-myc mRNA downregulation during myogenic differentiation".	1-10, 22 and 26

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/AU02/00351

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	9914346	AU	93193/98	CA	2304982	EP	1021549
WO	9529244	AU	22959/95	US	5587300		

END OF ANNEX